MAJOR ARTICLE

Epidemiologic Profile of Type-Specific Human Papillomavirus Infection and Cervical Neoplasia in Guanacaste, Costa Rica

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(See the editorial commentary by Winer and Koutsky, the article by Castle et al., and the brief report by Dunne et al., on pages 1787–9, 1808–16, and 1817–9, respectively.)

Background. Detailed epidemiologic studies of cervical type-specific human papillomavirus (HPV) infection in large populations are scarce.

Methods. We recruited a population-based cohort in Guanacaste, Costa Rica. Participants were interviewed, screened for cervical neoplasia, and tested for >40 HPV types by use of MY09/11 L1 consensus primer polymerase chain reaction. We estimated the risk factors for infection and the associations between type-specific HPV infections and cervical intraepithelial neoplasia (CIN) and cancer in 8514 sexually active women who had not undergone a hysterectomy.

Results. The overall HPV prevalence was 26.5%. The most common type was HPV-16 (3.6% of the population). HPV prevalence showed a U-shaped age-specific curve. Sexual behaviors were the main determinants of oncogenic and nononcogenic infections; age at first sexual intercourse was not independently associated with infection. Barrier contraceptive use was somewhat protective against infection. Oncogenic infections were strongly associated with risk of all grades of CIN and of cancer. Types 16, 18, and 58 were the most common in women diagnosed with CIN3 and cancer. Except for those that included HPV-16, multiple-type infections were associated with an increased risk (compared with that for single-type infections) of all grades of CIN and of cancer.

Conclusions. We confirmed the bimodal age pattern of HPV infection in Guanacaste and the sexually transmitted nature of both oncogenic and nononcogenic HPV types.

To date, >100 human papillomavirus (HPV) types have been identified, of which ~40 types are sexually transmitted and can infect the cervix. On the basis of laboratory and epidemiologic evidence, infections by a group of ~15 oncogenic HPV types are considered to be the necessary cause of cervical cancer and its precursor, cervical intraepithelial neoplasia (CIN) [1–4]. Most cervical infections—even by oncogenic types—are transient and cause either no detectable or mild pathological changes [5]. In some instances, when certain viral, host, or environmental cofactors are present, infections persist and can progress over the course of several years to CIN3 (precancer) and then possibly to invasive cervical cancer.

Detailed epidemiologic studies of HPV infection, CIN, and cancer are now addressing the natural history of individual HPV types, their differing roles in cervical carcinogenesis, and optimal strategies to prevent, via HPV screening and vaccination, the ⩾200,000 deaths caused by cervical cancer annually worldwide [3]. To examine population infection dynamics, large studies of HPV that are free from selection biases are needed.

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Such studies must distinguish individual HPV types, because each type independently causes only a fraction of cancer cases.

With these goals in mind, we are conducting a very large population-based study to investigate the natural history of HPV infection, CIN, and cancer in Guanacaste, a rural province of Costa Rica that had a high incidence of cervical cancer. Before the initiation of the Proyecto Epidemiológico Guanacaste (PEG), the region did not have effective cervical cytologic screening or state-of-the-art treatment. However, the beneficial effects of repeated rounds of effective screening and the ablative treatment of CIN2, CIN3, and cancer have possibly reduced the prevalence of the oncogenic HPV infections associated with these lesions. Thus, we have relied on our cross-sectional enrollment data, obtained before the initiation of treatment by PEG, to provide an unbiased examination of HPV infection and CIN and cancer in an entire population.

A previous interim study that was based on a portion of the enrollment data dealt with the prevalence of HPV in cervical lesions for a stratified sample of almost 3000 women and used an earlier polymerase chain reaction (PCR) method [6]. The number of women without detectable cervical abnormalities included in the stratified sample was limited. Moreover, we subsequently demonstrated that the analytic sensitivity of this PCR assay could be improved such that it would provide better epidemiologic data on HPV [7]. We have now completed baseline testing of the entire cohort of >9000 sexually active women using the improved PCR assay [7]. In the present analysis of 8514 sexually active women with an intact uterus and valid PCR results, we present a comprehensive analysis of the prevalence and determinants of individual and grouped HPV types and evaluate the relationships of CIN3 and cancer with single- versus multiple-type infections.

PARTICIPANTS, MATERIALS, AND METHODS

Study population. Detailed methods of this population-based study—approved by the institutional review boards of the National Cancer Institute (US) and Costa Rica—have been re-

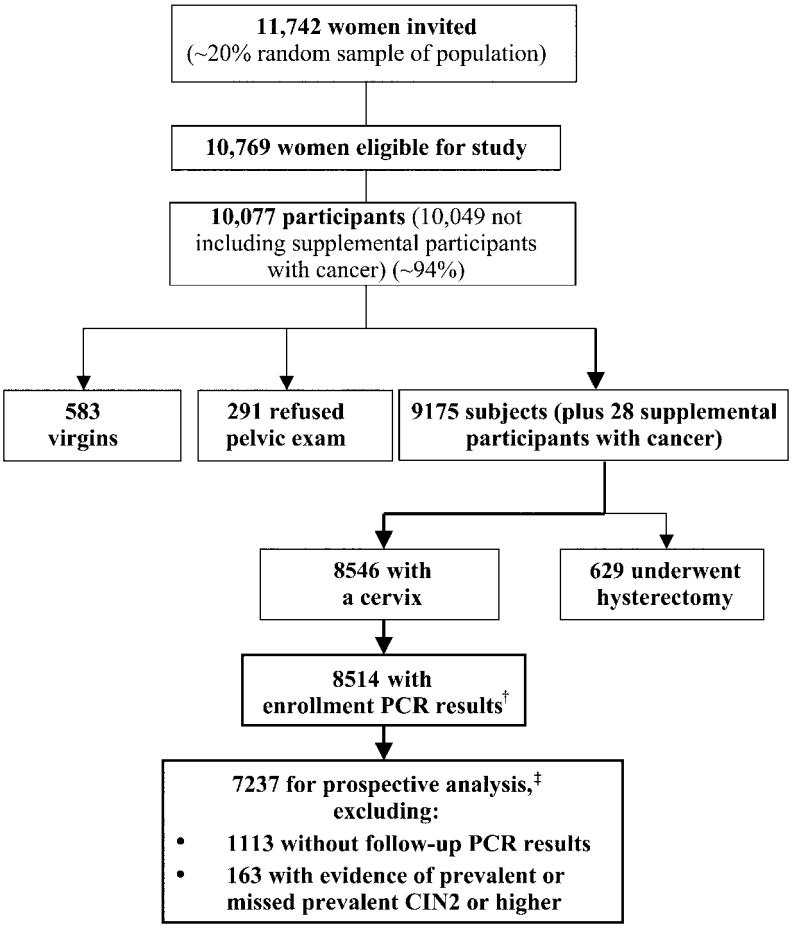

Figure 1. A summary of the participants of the Proyecto Epidemiológico Guanacaste included in the present analysis of type-specific human papillomavirus and in the accompanying prospective analysis in this issue of the *Journal of Infectious Diseases* [11]. †, included in the present analysis; ‡, included in [11]; CIN, cervical intraepithelial neoplasia; PCR, polymerase chain reaction.

Table 1. Prevalences of human papillomavirus (HPV) infection for specific types and categories of types (any type, oncogenic types, and nononcogenic types), both overall and by age group, for the Guanacaste cohort, excluding virgins, women who had undergone a hysterectomy, and supplemental patients with cancer ($n = 8513$).

Category, HPV type	No. of infections	Overall prevalence, % (95% CI)	HPV-positive participants, %	Prevalence by age group, %					
				<25 years ($n = 1041$)	25–34 years ($n = 2602$)	35–44 years ($n = 2074$)	45–54 years ($n = 1193$)	55–64 years ($n = 808$)	⩾65 years ($n = 795$)
Any type	2250	26.4 (25.5–27.4)	100.0	36.9	27.9	20.9	20.8	25.5	31.4
Oncogenic types									
Any oncogenic type	1170	13.7 (13.0–14.5)	52.0	24.4	15.4	9.8	9.7	10.8	13.6
16	302	3.6 (3.2–3.9)	13.4	6.5	4.3	2.4	2.4	2.3	3.1
18	112	1.3 (1.1–1.5)	4.9	3.0	1.2	0.8	0.8	1.4	1.5
26	16	0.2 (0.1–0.3)	0.7	0.1	0.2	0.2	0.3	0.2	0.3
31	121	1.4 (1.2–1.7)	5.4	3.0	1.7	0.7	1.0	1.0	1.3
33	59	0.7 (0.5–0.9)	2.6	1.2	0.5	0.6	0.5	0.7	1.0
35	36	0.4 (0.3–0.6)	1.6	0.6	0.5	0.4	0.2	0.3	0.8
39	69	0.8 (0.6–1.0)	3.1	1.6	1.1	0.5	0.5	0.3	0.5
45	66	0.8 (0.6–1.0)	2.9	1.1	0.8	0.9	0.5	0.6	0.6
51	166	2.0 (1.7–2.2)	7.4	3.9	1.8	1.5	1.4	1.9	1.8
52	135	1.6 (1.3–1.9)	5.9	3.8	1.6	0.9	0.9	1.2	1.6
56	75	0.9 (0.7–1.1)	3.3	1.4	0.9	0.5	0.7	1.0	1.1
58	170	2.0 (1.7–2.3)	7.5	5.6	2.2	1.1	0.8	1.4	1.6
59	31	0.4 (0.2–0.5)	1.4	1.0	0.4	0.3	0.0	0.4	0.1
66	68	0.8 (0.6–1.0)	3.0	1.8	1.1	0.4	0.5	0.2	0.6
68	29	0.3 (0.2–0.5)	1.3	1.2	0.2	0.2	0.3	0.4	0.1
73	38	0.5 (0.3–0.6)	1.7	1.0	0.4	0.2	0.2	0.5	0.8
AE2 (82 subtype)	31	0.4 (0.2–0.5)	1.4	0.4	0.3	0.3	0.5	0.5	0.5
Nononcogenic types									
Any nononcogenic types	1491	17.5 (16.7–18.3)	66.3	22.5	17.4	13.9	14.1	18.7	24.8
6	50	0.6 (0.4–0.8)	2.2	1.2	0.3	0.8	0.4	0.1	0.8
11	24	0.3 (0.2–0.4)	1.1	0.6	0.3	0.2	0.1	0.2	0.3
32	29	0.3 (0.2–0.5)	1.2	0.8	0.2	0.3	0.1	0.5	0.5
40	12	0.1 (0.06–0.2)	0.5	0.1	0.3	0.0	0.1	0.0	0.1
53	200	2.4 (2.0–2.7)	8.9	4.0	2.4	2.0	1.3	1.7	3.5
54	37	0.4 (0.3–0.6)	1.6	0.9	0.4	0.3	0.4	0.5	0.4
55	20	0.2 (0.1–0.3)	0.9	0.5	0.2	0.1	0.2	0.2	0.4
61	208	2.4 (2.1–2.8)	9.2	3.0	2.6	1.4	2.3	3.5	3.3
62	150	1.8 (1.5–2.0)	6.7	2.8	1.0	1.1	1.3	2.8	4.4
67	14	0.2 (0.08–0.3)	0.6	0.2	0.3	0.1	0.1	0.1	0.1
70	177	2.1 (1.8–2.4)	7.9	3.0	2.3	1.4	1.9	1.2	2.8
71	204	2.4 (2.1–2.7)	9.1	1.5	2.5	1.9	2.1	3.5	3.8
72	25	0.3 (0.2–0.4)	1.1	0.6	0.3	0.2	0.1	0.5	0.4
AE10 (74 variant)	15	0.2 (0.09–0.3)	0.7	0.2	0.1	0.2	0.1	0.5	0.1
81	103	1.2 (1.0–1.4)	4.6	2.2	1.0	0.8	0.9	1.2	2.0
83	100	1.2 (1.0–1.4)	4.4	1.1	0.8	1.0	1.0	2.2	2.4
84	58	0.7 (0.5–0.9)	2.6	1.0	0.8	0.6	0.3	0.6	0.8
85	59	0.7 (0.5–0.9)	2.6	1.0	0.5	0.3	0.9	1.2	1.0
89	21	0.3 (0.1–0.4)	0.9	0.3	0.3	0.1	0.2	0.6	0.0
Uncharacterized	250	2.9 (2.6–3.3)	11.1	3.2	3.1	2.6	2.6	2.5	4.2
Single-type infections	1552	18.2 (17.4–19.1)	69.0	20.8	19.9	15.8	15.6	17.7	20.6
Multiple-type infections	698	8.2 (7.6–8.8)	31.0	16.2	8.0	5.2	5.2	7.9	10.8

NOTE. The prevalence of an individual type includes detection of the type in a single- or multiple-type infection. CI, confidence interval.

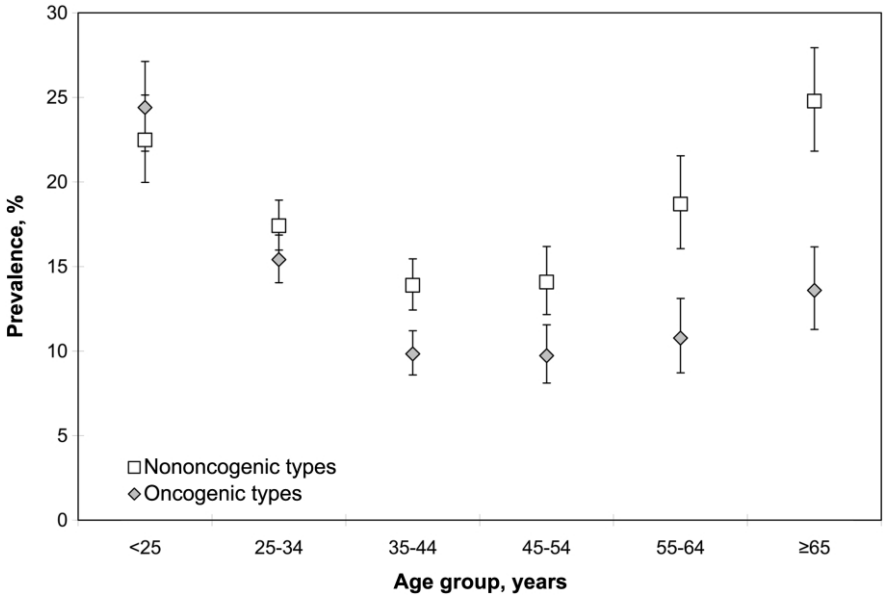

Figure 2. Prevalences of oncogenic human papillomavirus (HPV) types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and AE2 [82 subtype]) and nononcogenic HPV types (2, 6, 11, 13, 26, 32, 34, 40, 42–44, 53–55, 57, 61, 62, 64, 67–72, 74, AE10 [74 variant], 81–85, 89, and AE9), by age group. Bars indicate binomial exact 95% confidence intervals.

ported elsewhere [8, 9]. Briefly, after selecting a random sample of censal segments of this mainly rural population (240,000 inhabitants), we conducted a house-to-house enumeration of resident adult women (≥18 years old) and identified a target population of 11,742 potential participants. From June 1993 to December 1994, we invited these potential participants to visit local clinics for an appointment with our staff and recruited into the natural-history study those who agreed to participate and signed an informed-consent form. We obtained consent from all participants in accordance with the guidelines of the US Department of Health and Human Services.

Data and specimen collection. Trained interviewers administered a risk-factor questionnaire to 10,049 women (93.4% of the eligible participants) [8, 9]. Specially trained study nurses performed pelvic examinations on the sexually active women (9175 women were examined [97% of the eligible participants]); the examinations included cervical cell collection for cytologic and HPV DNA testing [8, 9]. After expert review of all cytologic and histologic material, we assigned a final enrollment diagnosis to each participant: normal screening results, equivocal lesions, low-grade lesions (including cytologic low-grade squamous intraepithelial lesions and histologic CIN1), CIN2, CIN3, or cancer. CIN2, CIN3, and cancer are routinely treated, but we focus here on CIN3 and cancer because of the regressive potential of CIN2. In addition to the women from the randomly selected cohort, we recruited all women who were diagnosed with invasive cervical cancer in Guanacaste during the period of enrollment into the study (hereafter, the "supplemental patients"). We included these supplemental patients in the analysis of the associations between HPV types and cancer; however, because we oversampled cancers, these supplemental patients were not included in the calculations of the population-based prevalences of HPV types.

Detection and genotyping of HPV. We previously conducted a methodological analysis to optimize our PCR assay [7]. On the basis of these results, we used MY09/M11 L1 consensus primer PCR (MY09/11 PCR) [10] with AmpliTaq Gold polymerase [7] for HPV DNA detection.

After amplification by this method, PCR products were analyzed by gel electrophoresis, transferred to nylon filters, and then hybridized overnight by use of radiolabeled generic probes for HPV (types 11, 16, 18, 51, 73, and 81 combined). Thereafter, PCR products positive for HPV by the radiolabeled generic probes were typed by use of dot-blot hybridization with biotinylated type-specific oligonucleotide probes for the following HPV types [7, 10]: 2, 6, 11, 13, 16, 18, 26, 31–35, 39, 40, 42–45, 51–59, 61, 62, 64, 66–74, AE10 (74 variant), 81–85, AE2 (82 subtype), 89, and AE9. To reduce cost and save time, we detected the rare types 2, 13, 34, 42–44, 57, 64, 69, 74, 82, and AE9 as a group by combining the dot-blot probes for those types. Hybridized probes were detected by use of a strepavidin–horseradish peroxidase conjugate (Amersham) and a commercial chemiluminescence detection kit (Amersham). A specimen was classified as HPV positive but uncharacterized if it tested positive for HPV DNA by the radiolabeled generic probe mix but was not positive for HPV DNA by the type-specific probe. Three experienced investigators interpreted each dot-blot result, and discrepancies were resolved by consensus.

Table 2. Risk factors for any nononcogenic human papillomavirus (HPV) type, for any oncogenic HPV type, and for HPV-16.

Category	No. of participants (%)	Nononcogenic		Oncogenic		HPV-16	
		OR (95% CI)	P_{Trend}	OR (95% CI)	P_{Trend}	OR (95% CI)	P_{Trend}
Age			.0005		<.0001		<.0001
<27 years (reference)	1523 (17.9)	1.0		1.0		1.0	
27–33 years	1867 (21.9)	0.7 (0.5–0.8)		0.6 (0.5–0.7)		0.5 (0.3–0.7)	
34–41 years	1803 (21.2)	0.5 (0.4–0.6)		0.4 (0.3–0.5)		0.3 (0.2–0.4)	
42–54 years	1717 (20.2)	0.5 (0.4–0.6)		0.3 (0.3–0.4)		0.3 (0.2–0.5)	
⩾55 years	1604 (18.8)	0.8 (0.6–1.1)		0.5 (0.3–0.6)		0.3 (0.2–0.6)	
Total	8514 (100.0)						
Education			.01		.006		.08
0 years (reference)	706 (8.3)	1.0		1.0		1.0	
1–3 years	1590 (18.7)	1.0 (0.8–1.3)		0.8 (0.6–1.1)		0.5 (0.3–0.8)	
4–6 years	3378 (39.7)	1.0 (0.8–1.3)		0.9 (0.7–1.2)		0.9 (0.5–1.4)	
7–9 years	1000 (11.8)	1.0 (0.7–1.3)		0.9 (0.7–1.3)		0.7 (0.4–1.3)	
⩾10 years	1832 (21.5)	1.3 (1.0–1.7)		1.2 (0.9–1.7)		1.1 (0.6–1.9)	
Total	8506 (100.0)						
No. of sex partners, lifetime			<.0001		<.0001		<.0001
1 (reference)	4596 (54.0)	1.0		1.0		1.0	
2	1837 (21.6)	1.4 (1.2–1.6)		1.5 (1.3–1.8)		2.0 (1.5–2.7)	
3	1079 (12.7)	1.8 (1.5–2.2)		1.9 (1.5–2.3)		2.6 (1.8–3.7)	
⩾4	1001 (11.8)	2.1 (1.7–2.5)		2.5 (2.0–3.0)		3.5 (2.4–5.1)	
Total	8513 (100.0)						
No. of sex partners, previous year			<.0001		<.0001		<.05
0 (reference)	1491 (17.5)	1.0		1.0		1.0	
1	6816 (80.1)	1.5 (1.2–2.0)		1.3 (0.9–1.8)		0.95 (0.5–1.7)	
⩾2	203 (2.4)	2.6 (1.7–3.9)		2.2 (1.4–3.4)		2.1 (1.0–4.5)	
Total	8510 (100.0)						
No. of pregnancies			.1		.9		.3
0 (reference)	426 (5.0)	1.0		1.0		1.0	
1	1092 (12.8)	0.8 (0.6–1.0)		0.9 (0.6–1.2)		1.0 (0.5–1.9)	
2	1419 (16.7)	0.7 (0.5–1.0)		0.9 (0.6–1.2)		1.1 (0.6–2.1)	
3	1376 (16.2)	0.7 (0.5–1.0)		0.9 (0.7–1.3)		1.6 (0.9–3.1)	
⩾4	4201 (49.3)	0.7 (0.6–1.0)		1.0 (0.7–1.3)		1.2 (0.6–2.2)	
Total	8514 (100.0)						
Sexual intercourse frequency, per month			.006		.1		.5
<2 (reference)	2169 (25.5)	1.0		1.0		1.0	
2–4	2515 (29.6)	0.9 (0.7–1.2)		1.0 (0.7–1.2)		0.8 (0.5–1.3)	
5–9	1888 (22.2)	0.8 (0.7–1.1)		0.8 (0.6–1.1)		0.8 (0.5–1.4)	
⩾9	1936 (22.8)	0.8 (0.6–1.0)		0.8 (0.6–1.1)		0.8 (0.5–1.3)	
Total	8508 (100.0)						

Use of any contraceptives								
Ever (reference)	6906 (81.1)	1.0		1.0		1.0		
Never	1607 (18.9)	1.2 (0.9–1.5)		1.2 (0.9–1.6)		0.7 (0.4–1.2)		
Total	8513 (100.0)							
Use of oral contraceptives			.002		.0002			.01
Never (reference)	3106 (36.5)	1.0		1.0		1.0		
Former	3655 (43.0)	1.3 (1.1–1.5)		1.2 (1.0–1.5)		1.0 (0.7–1.4)		
Current	1747 (20.5)	1.4 (1.1–1.7)		1.5 (1.2–2.0)		1.6 (1.1–2.4)		
Total	8508 (100.0)							
Use of barrier contraceptives			.01		.04			.8
Never (reference)	4748 (55.8)	1.0		1.0		1.0		
<5 years	3332 (39.2)	0.9 (0.7–1.0)		0.9 (0.8–1.0)		1.0 (0.7–1.3)		
⩾5 years	429 (5.0)	0.8 (0.6–1.1)		0.7 (0.5–1.0)		1.0 (0.5–1.9)		
Total	8509 (100.0)							
Tubal ligation								
No (reference)	7083 (83.2)	1.0		1.0		1.0		
Yes	1428 (16.8)	1.0 (0.8–1.2)		1.0 (0.8–1.3)		1.0 (0.7–1.6)		
Total	8511 (100.0)							
Ever had a venereal disease								
No (reference)	8183 (96.1)	1.0		1.0		1.0		
Yes	329 (3.9)	1.3 (1.0–1.8)		1.3 (0.9–1.8)		1.0 (0.5–1.7)		
Total	8512 (100.0)							
Smoking			.2		.6			.2
Never (reference)	7583 (89.1)	1.0		1.0		1.0		
Former	479 (5.6)	1.0 (0.8–1.3)		1.0 (0.7–1.3)		0.7 (0.4–1.3)		
Current	447 (5.3)	1.2 (0.9–1.5)		1.1 (0.8–1.5)		1.6 (1.0–2.4)		
Total	8509 (100.0)							
Ever had a Pap smear								
Yes (reference)	7441 (87.5)	1.0		1.0		1.0		
No	1065 (12.5)	1.2 (1.0–1.4)		1.1 (0.9–1.3)		1.0 (0.7–1.4)		
Total	8506 (100.0)							
Marital status								
Married, living together all year (reference)	6076 (71.4)	1.0		1.0		1.0		
Married, not living together all year	638 (7.5)	1.5 (1.2–1.8)		2.0 (1.6–2.5)		2.6 (1.8–3.7)		
Not married[a]	1800 (21.1)	1.8 (1.5–2.2)		1.8 (1.4–2.3)		1.3 (0.9–2.1)		
Total	8514 (100.0)							

NOTE. Multivariate logistic regression models were used to estimate the associations (odds ratios [ORs] and 95% confidence intervals [CIs]). The reference group was HPV-negative women. All risk factors are mutually adjusted for the others. Tests for trend (P_{Trend}), which are a measure of linear trend on the log scale, are also presented.

[a] Women who reported being separated, divorced, widowed, or single.

Statistical analysis. Of the 9175 sexually active women who had a pelvic examination at enrollment, we excluded 629 women who had undergone a hysterectomy and 32 women who did not have a PCR result. In total, we included 8513 women in the present analysis (figure 1).

We estimated the type-specific HPV prevalences and 95% confidence intervals (CIs) for the entire group and for each age group (<25, 25–34, 35–44, 45–54, 55–64, and ⩾65 years). To identify potential determinants of detection of HPV DNA, we calculated odds ratios (ORs) and 95% CIs, using logistic regression models adjusted for age in quintiles (<27, 27–33, 34–41, 42–54, and ⩾55 years). We included in multivariate models those variables found to be associated with risk of infection in age-adjusted models. We assessed dose-response relationships by treating ordinal variables as continuous (which assumes a linear trend) in the models (P_{Trend}) recognizing the limitations of such an assumption. We considered types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and AE2 (82 variant) to be oncogenic [4].

Age-adjusted logistic regression models were used to evaluate associations between single- and multiple-type infections with nononcogenic HPV types, oncogenic HPV types (excluding HPV-16), and HPV-16 infections and different grades of CIN and cancer. HPV-negative women were used as the reference group.

RESULTS

Overall HPV prevalence. Overall, 26.4% of the study participants had detectable HPV (25.4% after exclusion of women with CIN2, CIN3, or cancer) (table 1); 18.2% of the women were infected with a single type, and 8.2% of the women (31.0% of infected women) were infected with at least 2 HPV types. Oncogenic types and nononcogenic types were detected in 13.7% and 17.5% of the women, respectively.

HPV-16 was the most common oncogenic type detected (3.6% prevalence and 13.4% of all infections). HPV-61 was the most common nononcogenic type detected (2.4% prevalence and 9.2% of all infections).

Age-specific HPV prevalences. The age-specific prevalences of oncogenic HPV types decreased from 24.4% in the women <25 years old (figure 2) to 9.7% and 9.8% in the women 35–44 and 45–54 years old, respectively. A second peak in the prevalence of oncogenic HPV types reached a maximum of 12.6% in the women ⩾65 years old. Nononcogenic HPV types had an initial peak in prevalence of 22.5% in the women <25 years old, which decreased to 13.9% and 14.1% in the women 35–44 and 45–54 years old, respectively; a second peak of 24.8% occurred in the women ⩾65 years old.

Virtually all individual oncogenic HPV types were more common in the women <25 years old, with a decrease in prevalence in the women of intermediate ages and a second, minor peak in the older women (table 1 and figure 2). Most nononcogenic types had U-shaped age-specific prevalence curves, some with the highest prevalence in the women <25 years old (e.g., types 6, 32, 53, and 72) and others with the highest prevalence in the women ⩾65 years old (e.g., types 61, 62, 71, and 83).

Risk factors for prevalent HPV infection. The risk factors for prevalent HPV infection with oncogenic types and nononcogenic types, shown separately in table 2, were generally similar except when analyzed by age, which reflected the aforementioned differences in age-group prevalence. Increasing lifetime and recent (within the preceding year) numbers of sex partners were most strongly associated with being HPV positive for both HPV risk groups ($P_{\text{Trend}} < .001$).

Independent of the number of sex partners, the women who did not reside with their husbands all year were more likely to be infected with oncogenic (OR, 1.9 [95% CI, 1.6–2.4]) and nononcogenic (OR, 1.5 [95% CI, 1.2–1.9]) types than were the women who resided with their husbands all year. The unmarried women were also more likely to be infected with oncogenic (OR, 1.8 [95% CI, 1.4–2.3]) and nononcogenic (OR, 1.8 [95% CI, 1.5–2.3]) types than were the married women who resided with their husbands all year. Age at first sexual intercourse was not an independent risk factor for HPV infection once adjustments were made for the other factors in table 1.

Current oral contraceptive use was positively associated with oncogenic and nononcogenic infections, but barrier contraceptive use (primarily condom use) was somewhat protective against infection. The women who had been pregnant, regardless of the number, were less likely to be infected with nononcogenic types than were the women who had never been pregnant.

The findings for the women with multiple-type infections were similar to the findings for the women with single-type infections, as compared with HIV-negative women, but the associations with sexual-behavior variables appeared to be stronger for the women with multiple-type infections than for the women with single-type infections (data not shown). Barrier contraceptive use was strongly protective against multiple-type infections ($P_{\text{Trend}} = .0008$). Two or more recent sex partners and 2 and 3 or more lifetime sex partners were associated with HPV infection, compared with no recent sex partners and 1 lifetime sex partner, respectively, in both age groups (<45 and ⩾45 years) (table 3).

Prevalences of HPV types stratified by diagnosis. Table 4 presents the frequency of detection of HPV types by grade of CIN diagnosed at enrollment. Overall detection of HPV was 22.4% in the women with normal screening results, 42.1% in the women with equivocal lesions, 80.9% in the women with low-grade lesions, 81.8% in the women with CIN2, 93.2% in the women with CIN3, and 97.1% in the women with cancer.

Table 3. Multivariate logistic regression models to estimate the associations (odds ratios [ORs] and 95% confidence intervals [CI]) between sexual behavior variables and overall (any type) human papillomavirus (HPV) prevalence, stratified by age group (<45 and ⩾45 years old).

Recent no. of sex partners	Lifetime no. of sex partners					
	<45 years old			⩾45 years old		
	1 partner	2 partners	⩾3 partners	1 partner	2 partners	⩾3 partners
0 partners						
OR (95% CI)	1.0 (reference)	0.9 (0.6–1.6)	1.5 (0.9–2.5)	1.0 (reference)	1.2 (0.9–1.7)	1.7 (1.2–2.3)
No. of participants	171	100	101	516	277	325
Prevalence, %	5.4	8.3	7.7	36.7	44.2	42.7
1 partner						
OR (95% CI)	0.8 (0.5–1.2)	1.2 (0.8–1.9)	1.7 (1.1–2.5)	1.6 (1.1–2.3)	1.9 (1.2–2.9)	2.4 (1.6–3.7)
No. of participants	3017	1077	1066	890	348	418
Prevalence, %	94.6	89.1	81.0	63.3	55.5	54.8
⩾2 partners						
OR (95% CI)	NA	1.7 (0.8–4.1)	2.4 (1.4–4.0)	NA	8.9 (0.5–148.6)	7.0 (2.6–19.0)
No. of participants	NA	32	149	NA	2	19
Prevalence, %	NA	2.6	11.3	NA	0.3	2.5

NOTE. The reference group was HPV-negative women. ORs are adjusted for lifetime and recent nos. of sex partners, marital status, age, years of education, no. of pregnancies, sexual intercourse frequency, use of any contraceptives, use of oral contraceptives, use of barrier contraceptives, report of a tubal ligation, a history of venereal disease, smoking status, and ever having a Pap smear. NA, not available.

The percentage of multiple-type infections was lowest (25.8%) in the women with normal screening results, increased to almost 60% in the women with low-grade lesions, and decreased to 32.4% in the women with cancer; this pattern of peak or near-peak percentages of multiple types detected in women with low-grade lesions was consistently observed in all age groups (data not shown). There was a direct relationship between severity of diagnosis and the percentage of women infected with oncogenic types, ranging from 9.9% in the women with normal screening results to almost 90% in the women with cancer. In all abnormal diagnostic groups, HPV-16 was the most common type, increasing from 6.5% in the women with equivocal lesions to almost 50% in the women with CIN3 and cancer. All other oncogenic HPV types were detected in women with lesions of all grades, with a tendency (in order) of types 18, 58, 31, 33, 45, and 39 to be detected in the women with more-severe lesions. Types 51, 52, and 56 tended to be the predominant oncogenic types (apart from HPV-16) in the women with equivocal lesions, low-grade lesions, and CIN2.

Nononcogenic types were also common in the women with low-grade lesions, and the types detected in these women were generally the same as the ones that were commonly detected in the women with normal screening results—namely, types 53, 61, 62, 70, and 71. Types 53 and 70 were among the most common HPV types detected in the women with CIN2 (18.2%), although they were never found alone in the women with CIN3 and not at all in the women with cancer.

Prevalence of single- and multiple-type infections stratified by diagnosis. The age-adjusted associations between (1) diagnosis with different grades of CIN and cancer and (2) combinations of infections with single and multiple oncogenic and nononcogenic HPV types are shown in table 5. Infection with a single nononcogenic type moderately increased the risk of equivocal and low-grade lesions but did not significantly increase the risk of ⩾CIN2 and ⩾CIN3. In contrast, the presence of multiple nononcogenic types was significantly associated with higher risks of low-grade lesions and ⩾CIN2 but not ⩾CIN3. Infection with oncogenic types (even when HPV-16 was excluded) was strongly associated with an increased risk of low-grade lesions and ⩾CIN2; the ORs increased further for infection with combinations of oncogenic and nononcogenic types and for infection with multiple oncogenic types. Infection with HPV-16 was associated with the highest observed risks of ⩾CIN2 and ⩾CIN3. However, the ORs for infection with HPV-16 did not increase when additional types were also present.

DISCUSSION

By completing a substantial HPV typing effort in a large, representative population sample, we have strengthened some previous observations and revised other conclusions. Most important, the overall HPV prevalence reported in the present study was much higher than that reported in our previous study, a result of the increased analytic sensitivity in our revised HPV testing protocol. The major increase in HPV DNA detection

Table 4. Prevalences of human papillomavirus (HPV) infection for specific types and categories of types (any type, oncogenic types, and nononcogenic types), by severity of grade of cervical intraepithelial neoplasia (CIN).

Category, HPV type	Normal (n = 7459)	Equivocal (n = 727)	Low grade (n = 188)	CIN2 (n = 35)	CIN3 (n = 73)	Cancer[a] (n = 35)
Any type	22.4	42.1	80.9	81.8	93.2	97.1
Oncogenic types						
Any oncogenic type	9.9	26.1	68.6	74.6	89.0	88.6
16	2.2	6.5	15.4	36.4	49.3	45.7
18	1.1	1.7	3.2	3.6[b]	9.6[b]	17.1
26	0.2	0.4	0.0	0.0	1.4[b]	0.0
31	1.1	2.1	7.5	5.5	11.0	5.7[b]
33	0.5	1.2	3.7	5.5	4.1[b]	8.6[b]
35	0.2	1.2	3.2	5.5	1.4[b]	0.0
39	0.4	2.1	8.0	7.3[b]	2.7[b]	2.9
45	0.5	1.9	4.3[b]	3.6	2.7[b]	5.7
51	1.5	2.5	12.2	7.3	6.9[b]	2.9[b]
52	1.1	2.5	10.6[b]	10.9	5.5[b]	8.6
56	0.5	2.9	9.0	0.0	4.1	0.0
58	1.3	4.8	9.6	9.1	16.4	11.4
59	0.3	0.6	3.2[b]	0.0	2.7[b]	0.0
66	0.6	1.8	6.4	0.0	1.4[b]	2.9
68	0.2	1.4	1.1	0.0	1.4	0.0
73	0.3	1.1	3.2	7.3[b]	0.0	0.0
AE2 (82 subtype)	0.3	0.6	2.1[b]	1.8[b]	1.4[b]	0.0
Nononcogenic types						
Any nononcogenic type	12.5	16.0	12.2	7.3	4.1	8.6
6	0.4	1.5	4.3	1.8[b]	1.4[b]	0.0
11	0.2	0.6	2.1	1.8[b]	1.4[b]	2.9[b]
32	0.3	0.8	0.0	0.0	1.4[b]	0.0
40	0.1	0.4	1.6[b]	0.0	0.0	0.0
53	1.8	5.2	8.5	18.2	4.1[b]	0.0
54	0.3	0.7	1.6[b]	3.6[b]	4.1[b]	0.0
55	0.2	0.8	1.1[b]	0.0	0.0	0.0
61	2.3	3.0	6.4	3.6[b]	2.7[b]	2.9[b]
62	1.7	2.6	2.1	3.6[b]	2.7[b]	0.0
67	0.1	0.6	1.1	0.0	0.0	0.0
70	1.6	3.9	8.5	18.2	4.1[b]	5.7[b]
71	2.3	4.0	2.7[b]	1.8[b]	1.4	2.9[b]
72	0.2	0.8	0.5[b]	0.0	0.0	0.0
AE10 (74 variant)	0.1	0.7	0.0	3.6[b]	0.0	0.0
81	1.0	2.2	3.7	1.8[b]	2.7[b]	0.0
83	1.1	1.8	2.1	1.8[b]	2.7[b]	0.0
84	0.5	2.2	1.1[b]	1.8[b]	1.4[b]	0.0
85	0.7	0.6	1.1[b]	3.6	2.7[b]	0.0
89	0.2	1.0	0.5[b]	0.0	0.0	0.0
Uncharacterized	3.2	0.8	0.5	0.0	1.4	8.6
Single-type infections	16.6	25.9	33.5	38.2	52.1	65.7
Multiple-type infections	5.8	16.2	47.3	43.6	41.1	31.4
Infected participants with multiple-type infections	25.8	38.6	58.6	53.3	44.1	32.4

NOTE. Data are percentage of women. The prevalence of an individual type includes detection of the type in a single- or multiple-type infection.

[a] Includes supplemental patients with cancer.

[b] Indicated HPV type was never detected alone.

using our more sensitive MY09/11 PCR assay occurred in the population of women without detectable cervical abnormalities, who typically have lower viral loads than do women with detectable lesions [12]. Therefore, the increased sensitivity of our PCR assay mainly improved our epidemiologic inquiry of a previously undetected infection. As a consequence, the percentages of women found to have multiple-type infections increased, the typing of many previously unknown types was resolved, and the prevalence of HPV in the women with normal screening results increased dramatically. However, the risk estimates associating

Table 5. Age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for associations between categories of human papillomavirus (HPV) infection and severity of lesion.

Category	OR (95% CI)			
	Equivocal (n = 727)	Low grade (n = 188)	⩾CIN2 (n = 163)	⩾CIN3 (n = 108)
HPV negative (reference)	1.0	1.0	1.0	1.0
Nononcogenic				
Single-type infection with nononcogenic type	1.7 (1.4–2.2)	4.1 (2.4–7.1)	2.2 (0.8–6.1)	2.4 (0.5–12.0)
Multiple-type infection with nononcogenic types	2.4 (1.6–3.7)	12.1 (5.9–25.1)	10.2 (3.3–30.9)	6.9 (0.8–58.0)
Oncogenic (excluding HPV-16)				
Single-type infection with oncogenic type	2.8 (2.1–3.7)	17.8 (10.9–28.9)	27.1 (13.9–52.6)	34.8 (12.6–96.7)
Multiple-type infection with oncogenic and nononcogenic types	4.1 (2.8–5.9)	38.4 (22.8–64.9)	55.0 (26.9–112.3)	108.3 (39.6–296.0)
Multiple-type infection with oncogenic types	4.1 (2.6–6.5)	49.0 (27.7–86.6)	79.9 (37.1–172.0)	172.9 (61.2–485.3)
HPV-16				
Single-type infection with HPV-16	2.7 (1.7–4.5)	10.9 (4.7–25.3)	146.4 (78.6–273.0)	323.9 (131.1–798.3)
Multiple-type infection with HPV-16 and nononcogenic type(s)	5.1 (2.4–10.6)	51.3 (21.2–124.6)	144.1 (59.2–350.6)	83.7 (16.0–439.1)
Multiple-type infection with HPV-16 and other oncogenic type(s)	5.4 (3.0–9.7)	48.9 (23.9–100.0)	175.6 (77.8–396.8)	308.9 (101.9–936.2)

NOTE. CIN, cervical intraepithelial neoplasia.

HPV DNA detection with CIN and cancer decreased using the more sensitive PCR assay, because the prevalence of HPV increased primarily in the women with normal cytologic results rather than in the women with CIN or cancer. The difference in these 2 measurements highlights the difficulty of comparing the results of studies that use different PCR methods—which can be quite heterogeneous—and the continuing need for worldwide testing standards.

The increased prevalence observed with this more-sensitive PCR assay [7] (26.5% [95% CI, 25.5%–27.4%])—which was significantly greater than the previous prevalence estimate (16% [95% CI, 15%–18%]) [6] in the same population—appears to be real in light of 2 observations. First, infections detected exclusively by the current PCR assay were strongly and significantly associated with sexual risk factors (data not shown). Second, the percentage of infections with uncharacterized types (hybridization weakly positive for the general probe and negative for type-specific probes) was lower with this more-sensitive assay (data not shown).

As in most populations investigated [13–17], HPV-16 was found to be the most common in the present population. Nevertheless, its prevalence was relatively low in this general population (3.6%), which was composed primarily of women without evidence of HPV-induced lesions. Oncogenic types 58, 51, and 52 were also relatively abundant in this general population, followed by types 31 and 18. HPV prevalence surveys in Asia [18] and Africa [19] have reported relatively high HPV-58 prevalences, and HPV-52 is a common type in Asia [14, 18].

No PCR protocol is ideal for all types. For example, for the nononcogenic HPV types that we found to be common (e.g., HPV-53), sufficient information is not available for other populations, because the assays used in other evaluations have not routinely or optimally tested for these types [10, 20]. On the other hand, we note that there are some oncogenic types—such as HPV-68—that are less efficiently amplified by our PCR method than by other available methods (e.g., PGMY09/11); therefore, we, in turn, may have underestimated the prevalences of a few types in our population [10, 20].

In the present analysis, we have confirmed that the age distribution of HPV types in Guanacaste presents a U-shaped curve with peaks at the extremes of age, as previously reported in the stratified sample of the population [6]. Since we reported our original observation, some studies, particularly in areas of Latin America in which HPV prevalence is high, have observed a similar pattern [15, 21], whereas other studies in different areas have not [14, 16]. In Guanacaste, both oncogenic and nononcogenic HPV types displayed a U-shaped age-specific prevalence curve, although we observed a more-pronounced curve for nononcogenic types. We generally observed prevalence patterns for individual HPV types that were similar to those for the oncogenic and nononcogenic categories [11].

The lower second peak for oncogenic types, compared with that for nononcogenic types, could hypothetically reflect the tendency of oncogenic types to persist and induce overt disease, resulting in treatment and a censoring effect. However, at the initiation of the present study, cervical cancer rates were very high in Guanacaste [8]. The high rates had resulted largely from relatively infrequent, suboptimal Pap screening and lack of treatment of cancer precursors—although many Pap smears were performed, this population was essentially unscreened.

Theoretically, there are several possible explanations for a second peak in prevalence in older women, including (1) age-related sexual behavior of women or their male partners, (2) immune senescence leading to reactivation of latent infections or longer duration of new ones, (3) a cohort effect for sexual behavior or other risk factors, and (4) postmenopausal changes

that increase detection of HPV. We explore these patterns more extensively in the accompanying prospective study in this issue of the *Journal of Infectious Diseases* [11].

We made an effort to study differences in risk factors, especially in sexual behaviors, by age group as a possible explanation for the observed U-shaped age-specific HPV prevalence curves (data not shown). Although recent and lifetime numbers of sex partners were risk factors for infection in all age groups, in the present study, the older women were less likely to have >1 recent sex partner and had more lifetime partners than the younger women, suggesting that viral persistence better explains HPV prevalence in older women and that incident infection better explains HPV prevalence in younger women. This inference from cross-sectional data is examined further in our accompanying prospective analysis of HPV in this issue of the *Journal* [11].

The question of secular cohort changes in the prevalences of specific HPV types is a potentially important issue, because they could predict the types that will cause cancer in the future and, thus, the eventual need for changes in screening tests and vaccine formulations [4]. We observed a remarkable similarity in the proportions of specific HPV types among the HPV-positive women in the different age groups, suggesting that the same types have been prevalent for several decades in this essentially unscreened population.

As have other analyses [22, 23], the present analysis of behavioral risk factors for HPV infection provides convincing evidence for the sexual transmission of both oncogenic and nononcogenic HPV types. Age at first sexual intercourse was unrelated to HPV DNA positivity, once adjustments were made for number of sex partners.

The influence that nonsexual risk factors had on HPV prevalence was generally weak. Of note, we detected—concordant with the results of some studies but not of others—an increased number of oncogenic and nononcogenic HPV infections in current users of oral contraceptives, which may have resulted from the responsiveness of hormone-binding elements in the viral genome, the host immunologic response, or the response of the microanatomy of the cervix to hormonal influences [24]. Conversely, having been pregnant was negatively associated with HPV detection, especially for nononcogenic types; the number of pregnancies did not modify this effect. Other investigators have also reported a negative association between HPV detection and number of pregnancies [13], although multiparity is a risk factor for CIN3 and cancer in women infected with oncogenic HPV types. This seeming contradiction is not understood.

Several studies have investigated the potential protective effect of barrier contraceptives. A meta-analysis of the association between condom use and risk of cervical HPV infection and disease [25] indicated some evidence of protection against lesions but not against HPV infection. The present data from our population-based study in Guanacaste indicated a possible limited protection against infection, most notably against multiple-type infection, whereas, in a population-based study in Spain, a low-risk area, there was evidence that barrier contraceptives afforded stronger protection against infection [26].

The most common HPV type detected in women with advanced precursor lesions and cancer was 16, followed by 58, 18, and 31. Nononcogenic types, even when found in multiple-type infections, were not associated with a significant elevation in the risk of CIN3 or cancer. We note that some nononcogenic types were common in women with CIN2 but not in women with CIN3, suggesting that CIN3 and not CIN2 represents a truer precancerous diagnosis and a better surrogate for cancer. Typically nononcogenic types rarely cause CIN3 or cancer in the context of extreme influences by cofactors or poor host immune response. Alternatively, given that HPV testing is not perfectly sensitive and that multiple-type infections via a common route of transmission are often observed, these types might sometimes be proxies of infection by oncogenic types that are present but not detected.

The presence of multiple oncogenic types other than HPV-16 was associated with a higher risk of all grades of CIN and of cancer. Interestingly, with HPV-16 infection, the presence of other types did not confer added risk of $\geqslant$CIN2 or $\geqslant$CIN3.

In summary, the present analysis indicates that Guanacaste, in concordance with its historically high incidence of cervical cancer, has a relatively high prevalence of HPV infection in the general population, with a second peak in prevalence in older women. We have confirmed the sexual transmission of both oncogenic and nononcogenic HPV types to cervicovaginal mucosa and have demonstrated that barrier contraceptive methods may afford some protection. It has been shown that, in the present cohort, the most common HPV types in women with CIN3 and cancer are 16, 18, and 58, and vaccine formulations for use in Guanacaste should target these types.

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